## REMARKSARGUMENTS

Claims 1 and 30 remain in the application. Claims 1 and 30 have been amended. Reconsideration of this application, as amended, is respectfully requested.

Claims 1 and 30 have been amended to specify that the method is performed under aqueous conditions. Support for this amendment can be found at page 4, paragraph [0018] and page 5, paragraph [0023] of the specification. Claims 1 and 30 have been further amended to specify that the conjugate is suspended in or is soluble in aqueous solution. Support for this amendment can be found at page 4, paragraphs [0015], [0017], and [0018] of the specification. In claims 1 and 30, the term "protein" has been removed.

Claims 1 and 30 were rejected under 35 U. S. C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. This rejection is respectfully traversed for the following reasons.

The term "protein" has been removed from claims 1 and 30. Claims 1 and 30 recite that the conjugate is suspended or is soluble in water. For these reasons, this rejection should be withdrawn.

Claims 1 and 30 were rejected under 35 U. S. C. § 112, first paragraph, because the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate scope with the claims. This rejection is respectfully traversed for the following reasons.

In the method claimed herein, the steps a), b), and d) recited in claims 1 and 30 begin at paragraph [0097] and end at paragraph [0101] of the specification. The paragraphs discussing dimethylformamide are merely describing activation steps for (a) R-Phycoerythrin (paragraph [0091], (b) alkaline phosphatase (paragraph [0092], (c) alkaline phosphatase (paragraph [0093], (d) Anti-TSH $_{alpha}$ IgG antibody (paragraph [0094], (e) Anti-TSH $_{alpha}$ IgG antibody (paragraph [0095], and (f) oxidized agarose (paragraph [0096]), which are set forth in claims 1 and 30 at step c) as an optional step. (Step (c) of claims 1 and 30 recite

an activating step, if necessary, but none of the steps specifically recite the use of non-aqueous conditions.) In the steps shown paragraphs [0097] through [0101], inclusive, no solvent medium is expressly disclosed. For this reason, one of ordinary skill in the art would be expected to review the entire specification in order to determine what medium, if any, could be used to carry out the steps recited in claims of this application. According to paragraphs [0018] and [0023] of the specification:

> "Each step of the process, in fact the entire process, is preferably performed under aqueous conditions suitable to maintain the biological activity of an enzyme (e.g., bovine alkaline phosphatase)." (emphasis added)

> "The present inventive method is preferably performed under aqueous conditions, and more preferably performed entirely under aqueous conditions. The aqueous conditions are even more preferably selected so as to maintain the desired activity of the macromolecules that are conjugated. In an embodiment of the inventive method, the aqueous conditions are selected so as to maintain the catalytic activity of bovine intestinal alkaline phosphatase." (emphasis added)

Thus, it would be clear to one of ordinary skill in the art that an aqueous medium should be used to carry out the steps of the method recited in claims 1 and 30. For this reason, this rejection should be withdrawn.

Claims 1 and 30 stand rejected under 35 U. S. C. §102(e) as being anticipated by Yu et al. (U. S. 6,492,105). This rejection is respectfully traversed for the following reasons.

Yu et al., U. S. Patent No. 6,492,105 (hereinafter "Yu et al."), discloses polypeptide binding molecules prepared using solid phase peptide synthesis. Solid-phase synthesis begins at the carboxy-terminus of the putative polypeptide by coupling a protected amino acid to a suitable resin, which

reacts with the carboxy group of the C-terminal amino acid to form a bond that is readily cleaved later, such as a halomethyl resin, hydroxymethyl resin, aminomethyl resin, benzhydrylamine resin, or t-alkyloxycarbonyl-hydrazide resin. After removal of the $\alpha$-amino protecting group with, for example, trifluoroacetic acid in methylene chloride and neutralizing in, for example, TEA, the next cycle in synthesis is ready to proceed. The remaining $\alpha$-amino and, if necessary, side-chain-protected amino acids are then coupled sequentially in the desired order by condensation to obtain an intermediate compound connected to the resin. After the desired amino acid sequence has been completed, the intermediate polypeptide is removed from the resin support by treatment with a reagent, such as liquid HF and one or more thio-containing scavengers, which not only cleaves the polypeptide from the resin, but also cleaves all the remaining side-chain protecting groups. Following HF cleavage, the protein sequence is washed with ether, transferred to a large volume of dilute acetic acid, and stirred at pH adjusted to about 8.0 with ammonium hydroxide. Upon pH adjustment, the polypeptide takes its desired conformational arrangement.

Although Yu et al., at column 12, lines 61-63, discloses the presence of a covalent bond between an amino acid (which the Examiner has deemed analogous to the First Macromolecule recited in claim 1 of the present application) and another amino acid (which the Examiner has deemed analogous to the Second Macromolecule recited in claim 1 of the present application), Yu et al. is merely describing a conventional variant of synthesizing a polypeptide in which a preformed oligopeptide is coupled to a growing solid phase polypeptide chain in order to produce a longer, but still conventional, polypeptide chain. The synthesis described in Yu et al. is carried out under conditions that are substantially different from those conditions employed in the method of the present invention, with the result that the composition produced by the method described in Yu et al. is substantially different from the composition produced by the method of the present invention.

According to Yu et al., hydrazide-containing peptides were immobilized by facilitating hydazone bond formation, the GI-1 and -2 peptides were

immobilized via reductive amination using $NaCNBH_3$. See column 18, line 67 through column 19, line 3 of Yu et al. The nine peptides were evaluated by affinity chromatography for their ability to capture the REFACTO® polypeptide described in Example I, under specific binding and release conditions. See column 19, lines 9-11 of Yu et al. REFACTO® polypeptide is a recombinantly produced factor VIII-like polypeptide consisting of two segments of human factor VIII. See column 15, lines 3-10 of Yu et al.

The hydrazide-containing peptide can be considered to be the first macromolecule, while the recombinantly produced factor VIII-like polypeptide consisting of two segments of human factor VIII for which the hydrazide-containing peptide has binding affinity can be considered to be the second macromolecule. The method described in Yu et al. uses this binding affinity to isolate the factor VIII-like polypeptide from a complex mixture by binding the factor VIII-like polypeptide to a hydrazide-containing peptide immobilized on a solid support, and then releasing the factor VIII-like polypeptide by disruption of the bond between the factor VIII-like polypeptide and hydrazide-containing peptide. Yu et al. does not disclose or suggest that the bond to be disrupted is the bond that links the hydrazide-containing peptide to the solid support. In fact, the release conditions are specifically chosen in order to dissociate the hydrazide-containing polypeptide bound to the solid support from the factor VIII-like polypeptide, as pointed out in column 8, lines 15-44 of Yu et al. Further, in Example III, Yu et al. demonstrates that the solid support linked via hydrazone groups to several polypeptides shows binding and release of the factor VIII-like polypeptide. Yu et al. then shows that the solid support can be successfully reused without further exposure to the hydrazide-containing peptide, thereby showing that the peptide-hydrazone linkage to the solid support remains intact through the binding, elution, and washing steps described in Yu et al. See column 21, lines 12-61 of Yu et al.

In contrast, in the method of the present invention, the linkage targeted for disruption is the linkage between (a) the solid support and (b) the complex comprising the First Macromolecule and the at least one Second Macromolecule. For this reason, it is submitted that Yu et al. does not anticipate claims 1 and 30 of the present application.

Claims 1 and 30 stand rejected under 35 U. S. C. §102(e) as being anticipated by Schwartz (U. S. 2003/0013857 A1). This rejection is respectfully traversed for the following reasons.

Schwartz, U.S. Patent Application Publication 2003/0013857 A1 (hereinafter "Schwartz"), discloses modified solid supports that include solid supports that have been modified by reaction with a bifunctional reagent that possess a hydrazine or oxyamino group. These modified solid supports are useful in immobilization of biomolecules that possess or are modified to possess a carbonyl group. In one embodiment, aliphatic bifunctional hydrazide reagents are provided. These reagents include a cleavable bond for further manipulation. Cleavable bonds include, but are not limited to, acid cleavable, photocleavable and disulfide bonds.

In Schwartz, paragraph [0110] describes the use of cleavable linkers to create a drug-antibody conjugate, which is cleaved by physiological processes following endocytosis. Schwartz also refers to the use of cleavable disulfide linkages to isolate receptors following covalent linking between a ligand and a receptor.

Paragraph [0111] of Schwartz describes the use of bifunctional hydrazides to modify biomolecules or carriers in a single step. These modified aliphatic hydrazide molecules or carriers can be subsequently reacted with carbonyl containing biomolecules, drug, or other therapeutic or diagnostic reagent to form a hydrazone that can be cleaved following exposure to mild aqueous acid conditions.

Paragraph [0112] of Schwartz describes that solid supports such as beads, chromatographic supports or surfaces are modified with aliphatic hydazide reagents.

Paragraph [0118] of Schwartz describe solid supports modified with the reagents described therein so as to allow immobilization of biomolecules that contain a hydrazide group.

Paragraph [0147] of Schwartz describes hydrazino modified beads for forming stable hydrazones when reacted with molecules possessing carbonyl groups.

Paragraph [0172] of Schwartz describes hydrazones that are prepared from bifunctional hydrazine intermediates by treatment of these compounds with an aliphatic aldehyde or ketone to protect the hydrazine as its hydrazone.

Paragraph [0175] of Schwartz describes conjugates formed by the reaction of hydrazine-modified molecules with carbonyl-containing or carbonyl-modified molecules, hydrazine-modified molecules with carbonyl-modified surfaces or carbonyl-containing or carbonyl-modified molecules with hydrazine-modified surfaces.

Paragraph [0176] of Schwartz describes the modification of an organic soluble natural or synthetic molecule such as a hydrophobic peptide in organic solution by direct addition of the protected bifunctional hydrazino molecule to an organic soluble compound possessing an amine or thiol substituted moiety.

Paragraph [0177] of Schwartz describes the use of the reagents described therein to form crosslinks between a wide variety of molecules, such as, for example, protein-protein conjugates or protein-polymer conjugates.

Paragraph [0178] of Schwartz describes the crosslinking of conjugated biomolecules by incubation at ambient temperature of a hydrazine-modifed biomolecule and a carbonyl-modified biomolecule.

Paragraph [0179] of Schwartz describes immobilization of biomolecules to surfaces using a crosslinking couple by modifying the biomolecule with either a hydrazino, oxyamino, or a carbonyl moiety and contacting the modified biomolecule to a surface possessing its reactive partner, e.g., a hydrazino or oxyamino moiety for a carbonyl-modified biomolecule, or a carbonyl moiety for a hydrazino- or oxyamino-modified biomolecule.

Paragraph [0180] of Schwartz describes oxidation with sodium periodate of the carbohydrate domain of an antibody that is not involved in antigen binding to yield aldehyde moieties. The aldehyde moieties react directly with hydrazino- or oxyamino-modified surfaces to yield a stable covalent linkage in which a large percentage of the antibody active sites are available for antigen binding.

Paragraph [0181] of Schwartz describes that bisarylhydrazones formed from the reaction of an aromatic hydrazine and an aromatic carbonyl exhibit

more stability than do hydrazones formed from the reaction of aliphatic hydrazines and aliphatic carbonyls.

Example 17 of Schwartz exemplifies the coupling of hydrazino-modified BSA to oxidized dextran.

Although Schwartz provides a great number of descriptions of conjugations between biomolecules and solid supports with cleavable linkers, Schwartz does not disclose or suggest a method for preparing a conjugate in which (1) a first macromolecule is linked to a solid surface through a cleavable linker, (2) a second macromolecule is linked to the first macromolecule through a non-cleavable linker, and (3) the linker between the solid surface and the first macromolecule is cleaved, thereby (4) releasing the conjugate consisting of the first macromolecule linked to the second macromolecule by means of a non-cleavable linker. For these reasons, it is submitted that Schwartz does not anticipate claims 1 and 30 of the present application.

Claims 1 and 30 stand rejected under 35 U. S. C. §103(a) as being unpatentable over Merrifield (Reference AK of PTO form 1449). This rejection is respectfully traversed for the following reasons.

Merrifield, Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide, Journal of American Chemical Society, USA, 85, pp. 2149-2154 (July 1963) (hereinafter "Merrifield"), discloses the stepwise addition of protected amino acids to a growing peptide chain which was bound by a covalent bond to a solid resin particle. This provided a procedure whereby reagents and by-products were removed by filtration, and the recrystallization of intermediates was eliminated.

The method described in Merrifield depends on the attachment of the first amino acid of the chain to a solid polymer by a covalent bond, the addition of the succeeding amino acids one at a time in a stepwise manner until the desired sequence is assembled, and finally the removal of the peptide from the solid support. The intermediate peptides are purified, not by recrystallization procedures, but by dissolving away the impurities.

In the present invention, a First Macromolecule is attached to a Second Macromolecule, and so forth until a chain of macromolecules is formed. Because individual amino acids are not macromolecules, it is

submitted that Merrifield does not render claims 1 and 30 obvious to one of ordinary skill in the art.

The method described in Merrifield differs from the method claimed in the present application in the following respects:

(1) the nature of the materials being coupled;
(2) the nature of the chemical bonds being formed and broken;
(3) the nature of the solvents (liquid media) used;
(4) the reagents used in the reactions;
(5) the reaction conditions; and
(6) the character of the products produced

In Merrifield, the reactions are performed under conditions typical of organic synthesis, that is, with solvents such as, for example, dimethylformamide and methylene chloride. In fact, Merrifield states, with respect to "The Peptide-Forming Step":

> "Of the several solvents which were examined dimethylformamide was the best, methylene chloride was satisfactory, but dioxane, benzene, ethanol, pyridine and water gave very low yields and were not useful." (emphasis added)

See page 2150, column 2, lines 20-25 of "The Peptide-Forming Step" of Merrifield. Thus, it is clear that Merrifield teaches away from the use of water as a solvent that would be satisfactory for the peptide-forming step of his method. Claims 1 and 30 of the present application specify that the method be performed under aqueous conditions. To retain their biological activities, the macromolecules used in the present invention must remain in aqueous solvents, preferably under substantially physiological conditions. The organic synthetic conditions required for effective synthesis by means of the process described in Merrifield would denature most proteins, thereby destroying their biological activities and rendering the products useless for the purpose intended.

In Merrifield, the amino acid derivatives that are being coupled are not macromolecules. In the method of the present invention, the materials being coupled are macromolecules, which are **much larger** than the amino acid derivatives used in synthesis of peptides. Claim 30 specifies this feature. In view of the changes to claim 1 and to claim 30, it is submitted that Merrifield does not render claims 1 and 30 obvious to one of ordinary skill in the art.

In view of the foregoing, it is submitted that claims 1 and 30, as amended, are in condition for allowance, and official Notice of Allowance is respectfully requested.

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